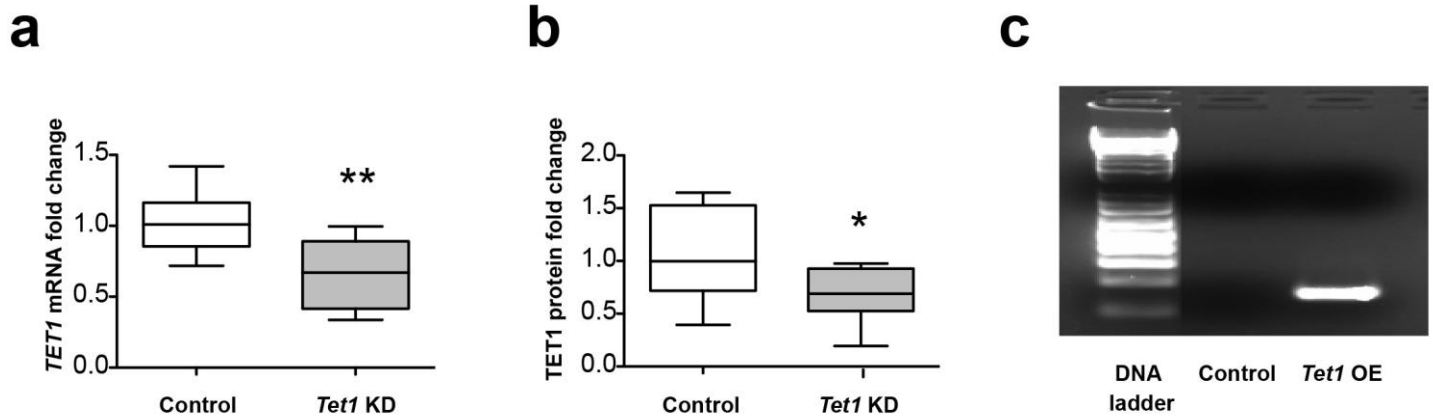**Supplementary Figure 1**

**Representative immunohistochemistry images and TET western blots.**

a, TET1 staining in NAc of mice treated with repeated cocaine or saline. A decrease in TET1 levels after cocaine is seen. **b**, Full length western blots for TET1, TET2, TET3, and β-actin. Saline (s) and cocaine (c) samples were loaded alternatively. The blue rectangles demonstrate the lanes cropped in **Figure 1c**. **c**, DAPI/GFP/DARPP-32 staining of a mouse brain section after viral injection. DAPI staining demonstrates intact brain structure of the injected hemisphere. GFP staining illustrates the spread of viral-mediated transgene expression in NAc. DARPP-32 immunohistochemistry indicates intact striatum structure after viral injection.

Supplementary Figure 2

**Validation of virally mediated *Tet1* shRNA knockdown and overexpression.**

a, qPCR indicates *Tet1* transcript levels are decreased after *Tet1* viral knockdown (KD) in NAc. Student unpaired *t*-test P=0.0026, t(18)=3.494. N=10 per group. **b**, Quantification by Western blotting confirms TET1 protein levels are also decreased after *Tet1* viral knockdown in this brain region. Student unpaired *t*-test, P=0.047, t(12)=1.805. N=8 per group. Box plots present, in ascending order, minimum sample value, first quartile, median, third quartile, maximum sample value. * indicates P<0.05, ** indicates P<0.01. **c**, DNA gel electrophoresis picture demonstrates the specific amplification of human *TET1* that is encoded by the viral overexpression (OE) construct in mouse NAc.
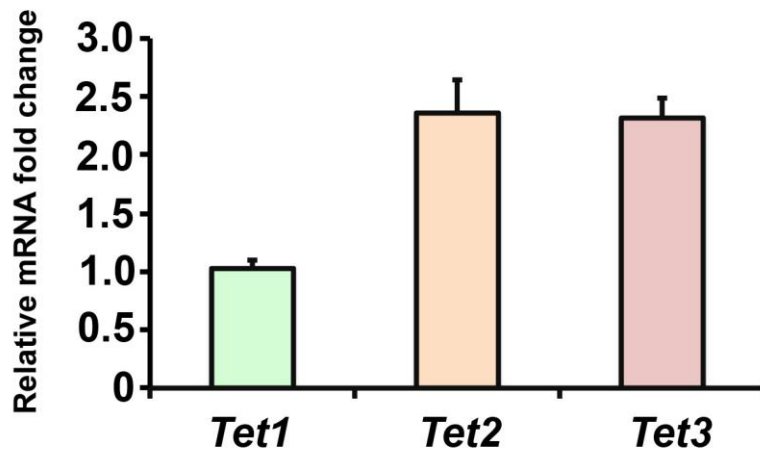
Supplementary Figure 3

**Relative expression of *Tet* mRNAs in adult mouse NAc.**

qPCR analysis demonstrates relative abundance of *Tet1*, *Tet2*, and *Tet3* transcripts in adult male mouse NAc. N=9 per group. Data are presented as mean ± SEM.
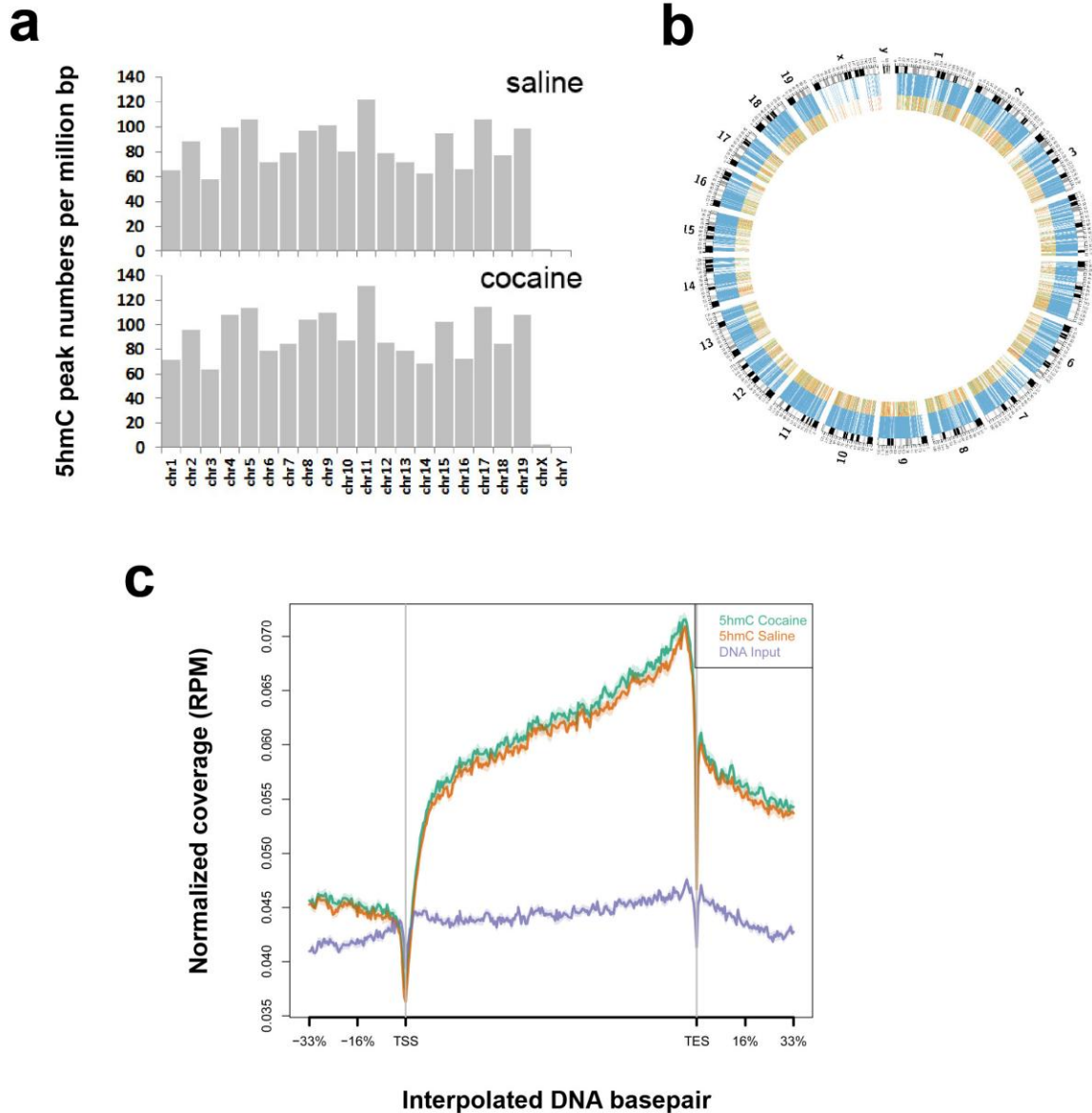
Supplementary Figure 4

**Distribution of 5hmC-enriched regions in NAc of saline- and cocaine-treated mice.**

a, Histogram of 5hmC peak counts (in numbers per million bp sequencing reads) of each chromosome in saline and repeated cocaine samples. **b**, Circular layout of 5hmC peaks under saline conditions in blue bars below the black-white ideogram along chromosomal coordinates. 5hmC differential sites after repeated cocaine are shown in yellow (red/green) bars of the inside circle. **c**, Coverage plot of 5hmC peak regions under saline (orange color) and cocaine (green color) conditions from transcription start sites (TSSs) to transcription ending sites (TESs), as well as up- and downstream regions, of genes genome-wide. The coverage of up- and downstream interpolated 33% sequence is also shown. DNA input trace is in purple color.
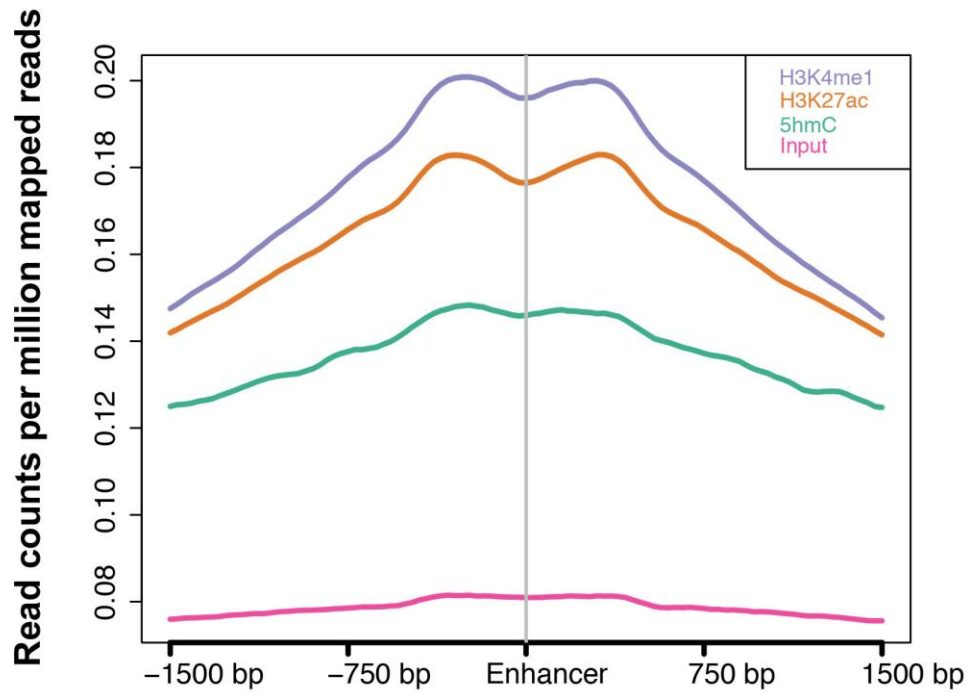
Supplementary Figure 5

Selective enrichment of H3K4me1, H3K27ac and 5hmC at enhancer regions.

ChIP-seq for H3K4me1 and H3K27ac as well as 5hmC-seq are plotted over the mouse brain enhancer regions defined by the ENCODE project (http://genome.ucsc.edu/ENCODE/). Coverage plot of H3K4me1 (blue color), H3K27ac (orange color), and 5hmC (green color) from 1500 bp up- and downstream of enhancer sites reveals their enrichment at enhancer regions as demonstrated by sequencing read counts while compared to input which has similar read counts as H3K4me3 (data not shown).
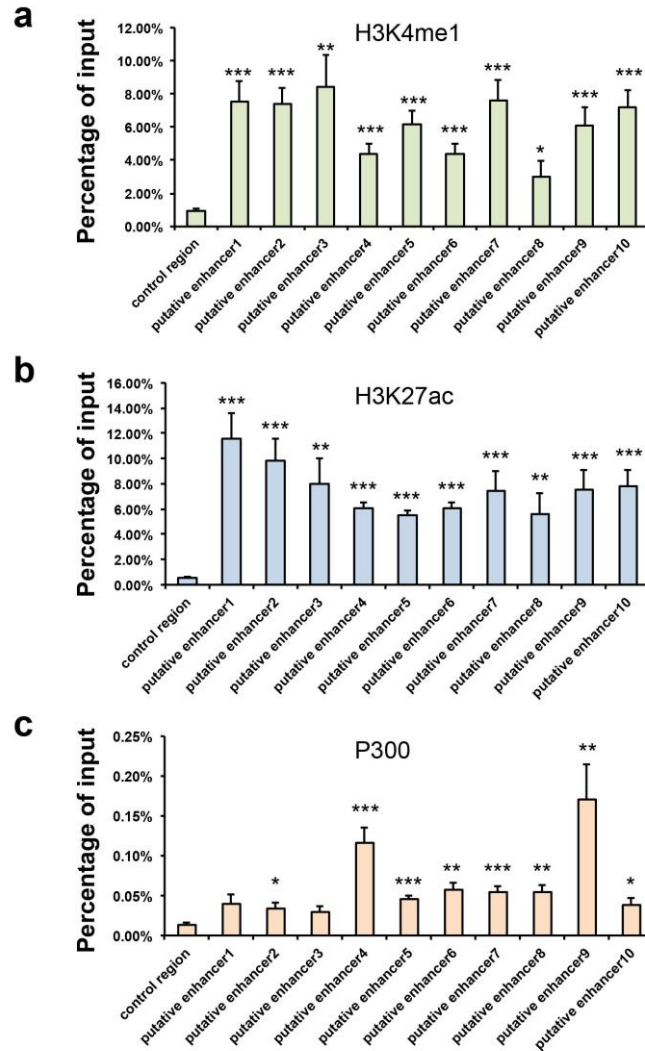
Supplementary Figure 6

ChIP-qPCR validation of putative enhancers.

a, H3K4me1 ChIP followed by qPCR from a separate cohort of animals display significant enrichments of H3K4me1 at 10 putative enhancer sites as predicted by H3K4me1 and H3K27ac ChIP-seq co-binding. Student's unpaired t-test for enhancers1 to 10: P=0.00008, t(14)=5.455; P=0.00001, t(14)=6.832; P=0.00143, t(14)=3.958; P=0.00005, t(14)=5.713; P=0.00001, t(14)=6.527; P=0.00005, t(14)=5.717; P=0.00013, t(14)=5.213; P=0.04619, t(14)=2.187; P=0.00050, t(14)=4.495; P=0.00004, t(14)=5.842. N=8/group. **b**, similar validation is observed for H3K27ac ChIP enrichment on the same putative enhancer sites. Student's unpaired t-test for enhancers1 to 10: P=0.00008, t(14)=5.507; P=0.00008, t(14)=5.481; P=0.00255, t(14)=3.665; P=2.94444E-08, t(14)=10.97; P=3.7615831E-09, t(14)=12.88; P=2.9862057E-08, t(14)=10.95; P=5.1980284E-04, t(14)=4.479; P=0.00624, t(14)=3.214; P=0.00042, t(14)=4.586; P=0.00007, t(14)=5.566. N=8/group. **c**, P300 ChIP demonstrates an enrichment at most of the 10 putative enhancer sites. Student's unpaired t-test for enhancers1 to 10: P=0.06760, t(11)=2.01; P=0.01612, t(11)=2.839; P=0.08876, t(11)=1.881; P=0.00036, t(11)=5.071; P=0.00026, t(11)=5.241; P=0.00123, t(11)=4.312; P=0.00091, t(11)=4.477; P=0.00155, t(11)=4.177; P=0.00820, t(11)=3.212; P=0.02076, t(11)=2.748. All putative enhancer bindings are compared to a non-putative enhancer control region at OCT4 gene promoter (-400bp to -550bp). All experiments are done under saline basal codition. The 10 randomly selected putative enhancer sites are chr8:124192000-124192600, chr12:32746400-32746800, chr13:49223800-49225000, chrX:53603400-53603600, chr13:74702000-74702200, chr18:38527400-38527600, chr12:118582600-118583200, chr17:25690800-25691000, chr9:61420400-61421200, chr3:90251000-90251400. Conrol n=6/group, enhancer n=7/group. Data are presented as mean ± SEM. * indicates *P*<0.05, ** indicates *P*<0.01, *** indicates *P*<0.001.
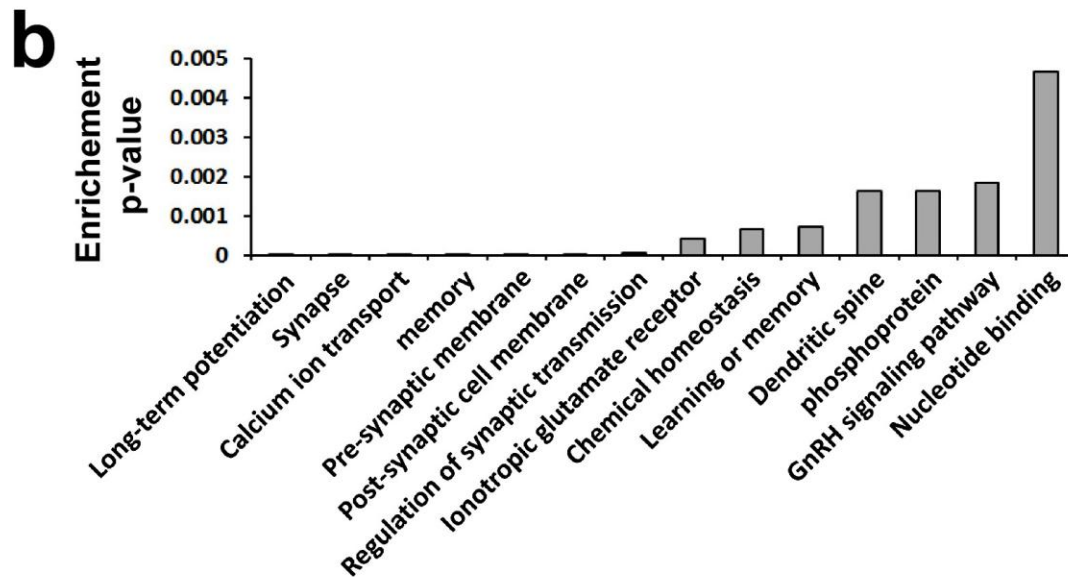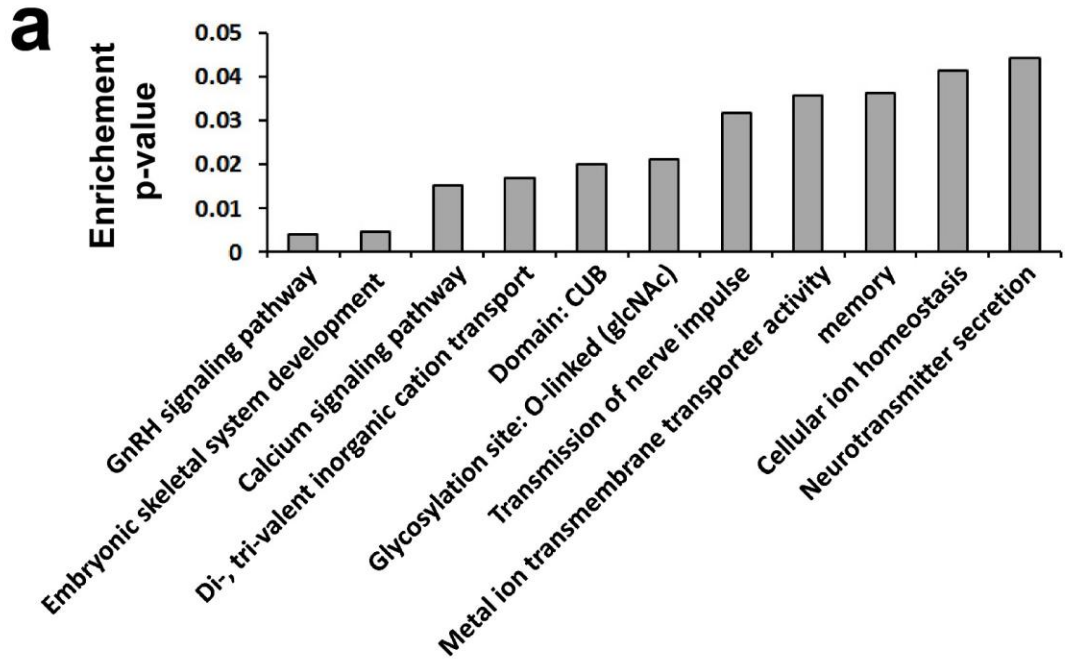
Supplementary Figure 7

Gene ontology of genes that show cocaine-induced changes by both 5hmC-seq and RNA-seq.

a, Gene ontology of the most significant enriched functional annotation groups of genes that display both mRNA induction (24 hr) and gene body 5hmC induction. **b**, Gene ontology of the most significant enriched annotation groups of genes that display both mRNA induction (4 hr after a cocaine challenge) and gene body 5hmC induction prior to the challenge dose of cocaine.
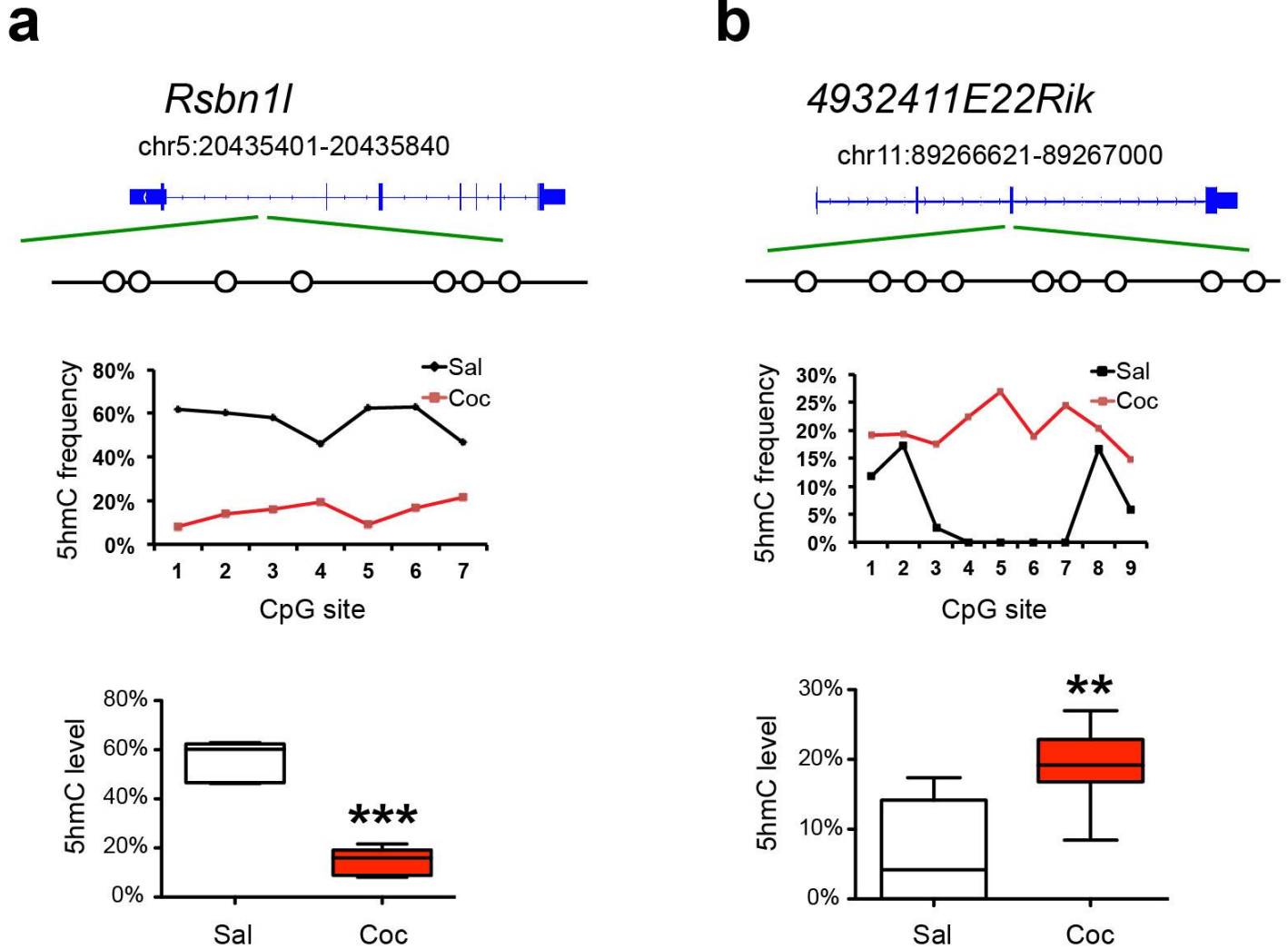
Supplementary Figure 8

oxBS-seq methodology validation.

Two loci with a significant increase (*4932411E22Rik*) or decrease (*Rsbn1l*) of 5hmC in NAc after repeated cocaine were selected from 5hmC-seq analysis. **a**, oxBS-seq confirmed that *Rsbn1l* shows decreased 5hmC frequency at all seven CpG sites. This decrease is statistically significant across the *Rsbn1l* locus. Student paired *t*-test, *P*=3.12E-08, t(6)=9.501. **b**, oxBS-seq confirmed that *4932411E22Rik* displays increased 5hmC frequency at most CpG sites tested. This increase is statistically significant across the *4932411E22Rik* locus. Student paired *t*-test, *P*=0.005, t(9)=3.687. N=1-2 biological replicates/condition. Box plots present, in ascending order, minimum sample value, first quartile, median, third quartile, maximum sample value. ** indicates *P*<0.01, *** indicates *P*<0.001.

Supplemetary Table 1. Sequencing data summary: quality control analysis.

sequencing sample	treatment	biological replicate	total_reads	unique_mapped_ reads	suppressed_multiple _mapped_reads	dup_reads	Normalized Strand Cross-correlation coefficient (NSC)	Relative Strand Cross-correlation coefficient (RSC)
5hmC campture seq	cocaine	1	53,506,844	29,050,796	3,232,508	8,080,990	1.131166	1.78968
5hmC campture seq	cocaine	2	55,484,874	29,892,092	4,374,056	12,366,589	1.125905	2.042943
5hmC campture seq	cocaine	3	59,113,826	33,305,418	3,764,785	10,190,299	1.135387	1.634807
5hmC campture seq	input	1	23,431,846	18,481,018	4,549,360	938,430	1.013705	0.5169814
5hmC campture seq	saline	1	62,494,817	35,041,044	5,207,006	10,096,461	1.086806	1.975856
5hmC campture seq	saline	2	53,710,729	30,011,210	3,914,698	9,352,472	1.1162	1.616004
5hmC campture seq	saline	3	56,310,244	26,278,274	3,242,848	5,712,005	1.117337	1.356048
H3K27ac ChIPseq	cocaine	1	229,709,230	175,096,234	4,727,926	22,581,443	1.065739	1.049676
H3K27ac ChIPseq	cocaine	2	218,846,752	172,922,245	4,822,984	20,979,535	1.063471	1.058677
H3K27ac ChIPseq	cocaine	3	223,097,857	172,359,715	5,004,955	18,202,651	1.063476	1.057878
H3K27ac ChIPseq	saline	1	217,535,912	155,757,197	4,363,260	15,368,259	1.06728	1.049129
H3K27ac ChIPseq	saline	2	228,476,479	177,862,421	5,097,915	20,477,853	1.063189	1.057762
H3K27ac ChIPseq	saline	3	217,635,175	137,125,779	3,909,253	13,269,955	1.071743	1.036415

sequencing sample	treatment	biological replicate	total_reads	mapped_reads	mitochondrial_RNA_ reads	rRNA_reads	mapping_rate	mitochondrial_RNA_ rate	rRNA_rate
4hr RNAseq	cocaine	1	38,962,356	36,766,764	2,239,550	0	94	6	0
4hr RNAseq	cocaine	2	40,926,637	39,574,235	2,394,015	0	97	6	0
4hr RNAseq	cocaine	3	40,723,069	39,414,193	2,416,842	0	97	6	0
4hr RNAseq	saline	1	40,568,820	38,278,617	2,398,875	0	94	6	0
4hr RNAseq	saline	2	40,091,363	38,871,668	1,329,654	0	97	3	0
4hr RNAseq	saline	3	40,441,794	39,276,870	2,033,176	0	97	5	0

Supplementary Table 5. Motif analysis of putative enhancer genes

category	motif name	e-value	distribution
cocaine state 4 & saline state 6	STAT1	3.00E-09	not centrally enriched
cocaine state 4 & saline state 6	Ascl2	5.50E-08	centrally enriched
cocaine state 4 & saline state 6	Zfp161	1.40E-07	centrally enriched
cocaine state 4 & saline state 6	HIF1A::ARNT	4.40E-05	centrally enriched
cocaine state 4 & saline state 6	Zbtb3	6.20E-04	centrally enriched
cocaine state 4 & saline state 6	TFAP2A	1.60E-03	centrally enriched
cocaine state 4 & saline state 6	Mafb	2.20E-03	centrally enriched
cocaine state 4 & saline state 6	Max	2.50E-03	centrally enriched
cocaine state 4 & saline state 6	Zic3/Zic1	4.00E-03	centrally enriched
cocaine state 4 & saline state 6	Gmeb1	9.60E-03	centrally enriched
cocaine state 5 & saline state 6	Myod1/Myf6	2.80E-03	not centrally enriched
cocaine state 5 & saline state 6	Sox10/Irf3	9.60E-03	not centrally enriched

Supplementary Table 7. List of primers used

gene name	forward primer	reverse primer	category
TET 1	GTCAGGGAGCTCATGGAGAC	CCTGAGAGCTCTTCCCTTCC	RT- qPCR
TET 2	GCAAGAGCTCTCAGGGATGT	AGGTCGCACTCGTACCAAAC	RT- qPCR
TET 3	CCAAGGCAAAGACCCTAACA	AGCAACTTCAGTGGCCAGAT	RT- qPCR
GAPDH	GGGTGTGAACCACGAGAAAT	GTCTTCTGGGTGGCAGTGAT	RT- qPCR
Human TET1	CAGCCTGTTCCCCGTTCTGG	AAAACCCGCATGAGGCTGTT	RT- qPCR
Human TET2	CAGCAGCCAATAGGACATGA	CCCTCAACATGGTTGGTTCT	RT- qPCR
Human TET3	CACCAAGAGTCTGCTGGACA	GGCCAGATCCCAAGTGAGTA	RT- qPCR
Human GAPDH	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG	RT- qPCR
Adcy1	CGTGGCTGAGGCTACTGAAG	GGTCACGTCATTGGACCATA	RT- qPCR
Itpr1	GGAGGACAAGGAAGCATTTG	TTGTGCCCTTTTCCAACTTC	RT- qPCR
Nxph4	TCAAGTTCTCGCTGTTGGTG	GGACGAATTGTGACGGAAGT	RT- qPCR
Hrk	AAACTGCTTGTCCCAACCAC	TGCTTACGGGCTCTTTGACT	RT- qPCR
Ntrk2	GCTCCTGCGACATCATGTG	TGTTCTTGCTGCTCTCATTGA	RT- qPCR
Akap6	AAGAGAAACCGCGACTGAAA	TCCTGGTATTCTCGGATTGC	RT- qPCR
Oct4 promoter	CTGTAAGGACAGGCCGAGAG	CAGGAGGCCTTCATTTTCAA	ChIP
putative enhancer 1	CTCTCCAGCCACTGTCTTCA	GCCTTGGTGTGAGAGAGGAT	ChIP
putative enhancer 2	AAATGCGGCCTTTGTCTCAG	AGAGATGCTGCCACTTGTCT	ChIP
putative enhancer 3	AGCTGGCCCTGATCATAGTC	CACACAGAACCAGAGACCCT	ChIP
putative enhancer 4	AGACCAAGCTGCTCATCTGT	TGCGGAATCTGAAGAGGACA	ChIP
putative enhancer 5	TGAAATCAAGGTCGCTAGCC	AAAGGACCTGCAGTTTCCCT	ChIP
putative enhancer 6	TGGGAGATAAAGGGCCTGTC	CCATCTCTCTAGCCCACGAG	ChIP
putative enhancer 7	TTTGCCTGCGTGTATGTCTG	AAGAGAGCTGGAGAGATGGC	ChIP
putative enhancer 8	AAAGCTGAGGTGGGGTGTTA	CAGGTGTGTGGCTGTAAAGG	ChIP
putative enhancer 9	AGTCCCATATCCCCAGGTCT	GATGGTTGCTTGGGACTTGG	ChIP
putative enhancer 10	AGGTGGAATTGAGCTTGGGA	AGGTGGCTTTGAAGGTCAGA	ChIP
Rsbn1l	TGTTTAATTTTGAATTTTTGTTTTTTG	TCTCTTATCTCTCTTCTTTATCTATCTCTA	BS
4932411E22Rik	TATTTTATTTTAAAGTAAATTGATGAAGT	ACTAATACTAATCTAATCTTCCCTACACTC	BS
Adcy1	TATGGGGATTTTGTTTTGATATATG	TATAAAAACCTCAACCCACATCTAC	BS
Itpr1	TTAGTTTTTTAGTAGTTGGGGTTAA	AAACATTTACCATAATTCCTATTTCC	BS
Nxph4	TAAGGGATTGGTAGGTAGTTGTTAG	CTATCCCCAACCCTTTAACTTAAA	BS
Hrk	GGTTTTGTTTATTAGTTTGGAATTTTA	ACATTAACAACAAACCAAAATACACTCT	BS
Akap6	TTTATGAGTGTTTGTATAAGGTGGG	ACAAAAAAATAAACTAAAAACCTTC	BS
Ntrk2	TTTTTTTAGTTTTTTTTGTTAGTTAATAAA	CACATCTCAATAATCAACCACATC	BS